

Serial No. 09/545,334  
Group Art Unit: 1635

Attorney Docket No. 0803

Version with Markings to Show Changes Made

## Regulated Expression of Genes in Plant Seeds

### WHAT IS CLAIMED IS:

1. A method for producing fertile, transgenic plants capable of the regulated expression of a cytokinin modulating gene in developing seeds comprising:  
introducing into plant host cells a genetic construct capable of preferential temporal and/or spatial expression of a cytokinin modulating gene in developing seed under conditions sufficient for the stable integration of the construct into the genome of said cells; and  
regenerating and recovering said fertile transgenic plants.
2. The method according to Claim 1 wherein the introduction of said construct is carried out by a process selected from the group consisting of electroporation, PEG poration, particle bombardment, silicon fiber delivery, microinjection, and Agrobacterium-mediated transformation.
3. The method according to Claim 2 wherein said process of introduction is particle bombardment.
4. The method according to Claim 2 wherein said process of introduction is Agrobacterium-mediated transformation.
5. The method according to Claim 1 wherein said genetic construct comprises a promoter directing temporal and/or spatial gene expression in plant seed operatively linked to a cytokinin modulating gene.
- ~~6. The method according to Claim 5 wherein said seed is from a dicotyledonous plant and said promoter is selected from the group consisting of bean  $\beta$ -phaseolin, napin,  $\beta$ -conglycinin and soybean-lectin-promoter.~~
7. The method according to Claim 5 wherein said seed is from a monocotyledonous plant and said promoter is selected from the group consisting of maize 15KD zein, 22KD zein, 27KD gamma zein, waxy, shrunken-1, shrunken -2, globulin-1, cim-1, end1, end 2, and gzw64a, and barley ltp2.
8. The method according to Claim 5 wherein said promoter directs embryo-preferred expression.
9. The method according to Claim 8 wherein said promoter is globulin-1.

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10. The method according to Claim 5 wherein said promoter directs endosperm-preferred expression.
11. The method according to Claim 10 wherein said promoter is 27KD gamma zein.
12. ~~The method according to Claim 1 wherein the cytokinin modulating gene is selected from the group consisting of genes encoding cytokinin biosynthetic enzymes, cytokinin catabolic enzymes, cytokinin catabolic enzyme antagonists and cytokinin biosynthetic enzyme agonists.~~
13. The method according to Claim 12 1 wherein said modulating gene encodes a cytokinin biosynthetic enzyme.
14. The method according to Claim 13 wherein said modulating gene encodes isopentenyl transferase.
15. ~~The method according to Claim 12 wherein said modulating gene encodes a cytokinin catabolic enzyme.~~
16. ~~The method according to Claim 15 wherein said modulating gene encodes cytokinin oxidase.~~
17. A fertile transgenic plant comprising a genetic construct stably integrated into the genome thereof, said construct capable of the temporal and/or spatial modulation of cytokinin levels in developing seed of said plant.
18. The plant according to Claim 17 wherein said genetic construct comprises a promoter directing temporal and/or spatial gene expression in plant seed operatively linked to a cytokinin modulating gene.
19. ~~The plant according to Claim 18 wherein said seed is from a dicotyledonous plant and said promoter is selected from the group consisting of bean  $\beta$  phaseolin, napin,  $\beta$ -conglycinin and soybean lectin promoter.~~
20. The plant according to Claim 18 wherein said seed is from a monocotyledonous plant and said promoter is selected from the group consisting of maize 15KD zein, 22KD zein, 27KD gamma zein, waxy, shrunken-1, shrunken-2, globulin-1, cim-1, end1, end2, and gzw64a, and barley ltp2.
21. The plant according to Claim 18 wherein said promoter directs embryo-preferred expression.
22. The plant according to Claim 21 wherein said promoter is globulin-1.
23. The plant according to Claim 18 wherein said promoter directs endosperm-preferred expression.
24. The plant according to Claim 23 wherein said promoter is 27KD gamma zein.

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25. ~~The plant according to Claim 17 wherein the cytokinin modulating gene is selected from the group consisting of genes encoding cytokinin biosynthetic enzymes, cytokinin catabolic enzymes, cytokinin catabolic enzyme antagonists and cytokinin biosynthetic enzyme agonists.~~
26. The plant according to Claim 25 18 wherein said modulating gene encodes a cytokinin biosynthetic enzyme.
27. The plant according to Claim 26 wherein said modulating gene encodes isopentenyl transferase.
28. ~~The plant according to Claim 25 wherein said modulating gene encodes a cytokinin catabolic enzyme.~~
29. ~~The plant according to Claim 28 wherein said modulating gene encodes cytokinin oxidase.~~
30. An isolated recombinant DNA comprising a genetic construct that comprises a promoter directing temporal and/or spatial gene expression in plant seed operatively linked to a cytokinin modulating gene.
31. ~~The DNA according to Claim 30 wherein said seed is from a dicotyledonous plant and said promoter is selected from the group consisting of bean  $\beta$ -phaseolin, napin,  $\beta$ -conglycinin and soybean lectin promoter.~~
32. The DNA according to Claim 30 wherein said seed is from a monocotyledonous plant and said promoter is selected from the group consisting of maize 15KD zein, 22KD zein, 27KD gamma zein, waxy, shrunken-1, shrunken -2, globulin-1, cim-1, end1, end2, and gzw64a, and barley ltp2.
33. The DNA according to Claim 30 wherein said promoter directs embryo-preferred expression.
34. The DNA according to Claim 33 wherein said promoter is globulin-1.
35. The DNA according to Claim 30 wherein said promoter directs endosperm-preferred expression.
36. The DNA according to Claim 35 wherein said promoter is 27KD gamma zein.
37. ~~The DNA according to Claim 30 wherein the cytokinin modulating gene is selected from the group consisting of genes encoding cytokinin biosynthetic enzymes, cytokinin catabolic enzymes, cytokinin catabolic enzyme antagonists and cytokinin biosynthetic enzyme agonists.~~
38. The DNA according to Claim ~~37~~ 30 wherein said modulating gene encodes a cytokinin biosynthetic enzyme.

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39. The DNA according to Claim 38 wherein said modulating gene encodes isopentenyl transferase.
- ~~40. The DNA according to Claim 37 wherein said modulating gene encodes a cytokinin catabolic enzyme.~~
- ~~41. The DNA according to Claim 40 wherein said modulating gene encodes cytokinin oxidase.~~
42. Host plant cells having stably introduced therein the genetic construct of Claim 30.
43. A method for improving stress tolerance and yield stability in plants in need thereof comprising stably introducing into cells of said plants a genetic construct capable of preferentially expressing cytokinin modulating genes during the lag phase of plant seed development and regenerating and recovering plants from said cells.
44. The method according to Claim 43 wherein said preferential expression occurs from about -14 to about 25 days after pollination.
45. The method according to Claim 43 wherein said preferential expression occurs from about 4 to about 21 days after pollination.
46. The method according to Claim 43 wherein said preferential expression occurs from about 4 to about 12 days after pollination.
47. The method according to Claim 43 wherein said preferential expression occurs from about 8 to about 12 days after pollination.